Willem P.C. Stemmer

Application No: 10/623,036

Filed: July 18, 2003

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Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

- 1. (Currently amended) A method for introducing one or more mutations into a template double stranded polynucleotide, wherein the template double-stranded polynucleotide has been cleaved into double stranded random-fragments of a desired size, selecting or screening a library of recombinant proteins to identify a recombinant protein having a desired functional property, said method comprising:
- a) randomly fragmenting a template double-stranded DNA into a plurality of double-stranded fragments of a desired size;
- ab) adding to the resultant population of double-stranded fragments one or more single or double-stranded oligonucleotides, wherein said oligonucleotides comprise an area of identity and an area of heterology to the template polynucleotide;
- bc) denaturing the resultant mixture of double-stranded random fragments and oligonucleotides into single-stranded fragments;
- ed) incubating the resultant population of single-stranded fragments with a polymerase under conditions which result in the annealing of said single-stranded fragments at regions of identity, to form pairs of annealed fragments, said areas of identity being sufficient for one member of a pair to prime replication of the other thereby forming between the single-stranded fragments and formation of a mutagenized double-stranded polymucleotides DNA molecules; and
- de) repeating steps (bc) and (ed) a desired number of times, wherein repeated step c) comprises denaturing the mutagenized double-stranded DNA molecules from step d) of the previous cycle to form a library of mutagnized double-stranded DNA molecules;
- f) expressing a library of recombinant proteins from the library of mutagenized double-stranded DNA from step e); and
- g) selecting or screening the library of recombinant proteins to identify a recombinant protein with a desired functional property.
- 2. (Original) The method of Claim 1 wherein the concentration of a specific double-stranded fragment in the mixture of double-stranded fragments is less than 1% by weight of the total DNA.

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- 3. (Original) The method of Claim 1 wherein the number of different specific double-stranded fragments comprises at least about 100.
- 4. (Original) The method of Claim 1 wherein the size of the double-stranded fragments is from about 5 bp to 5 kb.
- 5. (Currently amended) The method of Claim 1 wherein the <u>size of the mutagenized</u> double-stranded <u>DNA molecules in the library of size of the mutagenized-template</u> double-stranded polynucleotide <u>DNA molecules comprises</u> is from about 50 bp to 100 kb.

Claims 6-32 (Cancelled)

- 33. (New) The method of Claim 1, wherein the template double-stranded polynucleotide encodes a wild-type protein.
 - 34. (New) The method of Claim 1, wherein the polymerase is Taq.
 - 35. (New) The method of Claim 1, wherein the polymerase is Klenow polymerase.
- 36. (New) The method of Claim 1, wherein the template double-stranded DNA is from 50 bp to 50 kb.
- 37. (New) The method of Claim 1, wherein the size of the double-stranded fragments is from about 10 bp to 1000 bp.
- 38. (New) The method of Claim 1, wherein the size of the double-stranded fragments is from about 20 bp to 500 bp.